

Cryptic Species, Species Delimitation, and Taxonomic Practice in the Homosporous Ferns

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Biologists generally agree that species are to be delimited on the basis of genetic discontinuities. The two species concepts that depend on such discontinuities to delimit species are the biological and the evolutionary species concepts. A biological species is a group of interfertile populations that is reproductively isolated from other such groups and that occupies a specific niche in nature (following Mayr, 1982). An evolutionary species is a single lineage of ancestor-descendant populations that maintains its own identity from other such lineages, that fits into its own ecological niche, and that has a unique evolutionary history (Simpson, 1961; Grant, 1981; Wiley, 1981). It thus differs from the biological species concept in that it is equally applicable to both sexually and asexually reproducing organisms. Under both the biological and the evolutionary species concepts, genetic discontinuities between sister species are thought to arise stochastically following speciation. It is assumed that as time passes, the two diverge progressively in a suite of morphological, physiological, and ecological attributes.

Although most botanists espouse an evolutionary species concept in their theoretical writings, in their classifications they often recognize only species that have distinctive structural characters by which the taxa can be identified. In so doing, they are employing the morphological species concept of their predecessors. Thus a conflict arises between theory and practice.

As long as evolutionary species are structurally well differentiated, there is usually good agreement between species defined using either biological or morphological criteria. After all, a morphological species is "an inference as to the most probable limits of the biological species" (Dobzhansky, 1951), and the gaps by which such species are recognized are presumed to have arisen along with the reproductive isolation between them. But in the case of cryptic species, morphologically similar or identical natural populations that are reproductively isolated (Mayr, 1970), a species defined using morphological criteria comprises two or more genetically isolated evolutionary species.

The practical consequences of unrecognized cryptic species range from a simple underestimate of diversity in a study group to effects of substantial economic importance. For instance, the parasitic Hymenopteran species *Aphytis maculicornis*, imported into California to control olive scale, has been found by Hafez and Doutt (1954) to comprise at least three cryptic species. Of these, only one has proven effective as a biological control agent (DeBach, 1969). Another example, from human epidemiology, is that of the European mosquito

Anopheles maculipennis. This species was for a long time considered the vector of malaria among human populations in Europe. More recent studies have shown that six cryptic species occur within *A. maculipennis*, only two of which (*A. labranchiae* and *A. sacharowi*) carry malaria among humans (Mayr, 1970).

In systematic research on the ferns, new data from morphometrics, cytogenetics, and electrophoresis are greatly improving our ability to resolve problems in taxonomically difficult groups. In the process of resolving them we are discovering cryptic species in many lineages where they had previously been unsuspected. Satisfactory taxonomic treatment of these cryptic species depends on a resolution of the conflict between the evolutionary species concept we embrace in theory and the morphological species concept we commonly employ in practice.

CHARACTERIZATION AND RECOGNITION OF CRYPTIC SPECIES

Cryptic species (also called sibling species by some authors, e.g., Mayr, 1963) were defined by Stebbins (1950) as "... population systems which were believed to belong to the same species until genetic evidence showed the existence of isolating mechanisms separating them." Grant (1981) defined them as "... good biological species which are virtually indistinguishable morphologically." Wiley writes: "Cryptic species ... are species that cannot be diagnosed by morphology, but that act as independent evolutionary lineages in nature" (Wiley, 1981). Although each author's definition reflects his particular approach to the species question, several elements are common to all three. Cryptic species have the following characteristics:

1. They are poorly differentiated morphologically.
2. They represent distinct evolutionary lineages because they are reproductively isolated.
3. They have historically been misinterpreted as members of a single species.

These characteristics provide a set of criteria by which putative cases can be evaluated.

A classic example from the genus *Drosophila* illustrates application of these three criteria to cryptic species of fruit flies (Dobzhansky and Epling, 1944; Dobzhansky, 1951). Populations of *D. pseudoobscura* and *D. persimilis* were originally treated as one species until researchers observed hybrid sterility barriers between them in laboratory cultures. Once reproductive isolation of the two species was recognized, subtle differences between them were found in attributes of the wings and male genitalia. A series of subsequent studies identified additional differences in physiology, behavior, and chromosome morphology.

In the following discussion we use the criteria of subtle morphological differentiation, reproductive isolation, and historical confusion to evaluate the evidence for cryptic species in several problematic genera of ferns. Reproductive isolation is used as the primary criterion for determining whether or not two

morphologically similar populations represent cryptic species. In the absence of such evidence, the alternative explanation, that the populations are merely infraspecific variants, is favored. Discussion of cryptic species in plants often centers on polyploids and their diploid progenitors (e.g., Grant, 1981; see also Barrington et al., 1989). This paper, however, addresses the conceptually different problem of cryptic species at the same ploidy level. We also explore the factors that have obscured species boundaries in the ferns and demonstrate how new systematic methods are increasing our ability to define those boundaries. Finally we consider the practical problems posed by cryptic species to fern systematics and taxonomy.

RECENTLY RESOLVED CRYPTIC COMPLEXES IN THE FERNS

A. The *Adiantum pedatum* complex

Cryptic species have recently been documented within the *Adiantum pedatum* (maidenhair fern) complex in eastern North America. They are the typical maidenhair of rich deciduous woods and the serpentine maidenhair, a small serpentine endemic with fastigiate axes. Both are diploid (Paris, 1986; Paris & Windham, 1988).

The serpentine diploid has traditionally been interpreted as an infraspecific variant of the typical maidenhair (Fernald, 1905; Cody, 1983). Other authors have not recognized the taxon, presumably because many of the characters used to diagnose it are susceptible to environmental modification (Fernald, 1905; Wylie, 1949; Paris, unpubl. data) and because for most key characters it overlaps with the typical maidenhair (see Lellinger, 1985).

Isozyme data, however, have demonstrated that the diploids are well differentiated genetically (Paris & Windham, 1988); indeed, at several loci, the two taxa share no alleles in common. The average Nei's genetic identity for populations of the serpentine and non-serpentine maidenhairs is only 0.495, a value comparable to those available for congeneric fern species (Haufler, 1987) and quite low as compared with angiosperm congeners ($\bar{I} = 0.67$, Crawford, 1983).

Isozyme electrophoresis has also permitted the detection of still another maidenhair—a tetraploid population at Belvidere Mountain in Vermont. Additivity in the tetraploid of isozyme markers for the diploid taxa showed that the tetraploid was derived from a hybrid between the serpentine and the typical maidenhairs. Gametophyte progeny tests indicated that heterozygosity is fixed in the allotetraploid. Non-pairing of the two genomes provided further evidence that the serpentine and the typical maidenhair ferns represent distinct species (Paris & Windham, 1988).

With a new understanding of these entities and their relationships, morphological characters were reevaluated in a discriminant analysis. Three morphologically distinct groups corresponding to the taxa emerged, with the tetraploids occupying an intermediate position between their diploid progenitors (Paris & Windham, 1988).

It is now evident that the serpentine and the typical maidenhair ferns differ in a number of morphological characters, but that differences between them were obscured by the unrecognized tetraploid and by phenotypic plasticity. Thus they represent a good example of cryptic species: as was the case with Dobzhansky's *Drosophila*s, once the isolation of the taxa was perceived, morphological characters were found to differentiate them.

B. *Botrychium* subg. *Botrychium*

Additional examples of cryptic species are available from recent systematic work in *Botrychium* subg. *Botrychium* (moonworts) in western North America (e.g., Wagner & Wagner, 1983a, 1983b). *Botrychium* is a taxonomically difficult genus in a number of respects: first, in subgenus *Botrychium* many of the plants are small and so are overlooked by collectors. In consequence, they tend to be poorly represented in herbaria. Furthermore, morphological characters are difficult in subgenus *Botrychium*: not only are they quite subtle, requiring careful definition and comparison, but also they are readily influenced both by the age of the sporophyte and by the habitat in which it grew. The susceptibility of *Botrychium* species to environmental modification is best demonstrated by variation within *B. simplex*, the typical form of which is moderately dissected and occurs in dry upland fields. In deep forests and at bog edges, a delicate, relatively undissected form occurs ("var. *tenebrosum*" of Clausen), whereas a robust and ternately dissected form (Milde's "var. *compositum*") is found in low, moist meadows.

In some *Botrychium* taxa, morphological variation is genetically controlled. A well-known example is provided by *B. dissectum* forma *dissectum* and *B. dissectum* forma *obliquum* (subg. *Sceptridium*), morphologically distinct entities that maintain their distinctive characteristics when growing side by side. These forms were originally thought to be species, then varieties. When it was recognized that the two are fully interfertile and that a range of intermediates exists, they were relegated to forms (Fernald, 1921).

Given such complex and apparently inconsistent patterns of variation in *Botrychium*, how can sound taxonomic judgments be made? Common garden experiments, useful in addressing such questions in other taxa, are problematic in *Botrychium* because complex and sensitive mycorrhizal relationships make them difficult to grow. Also, botrychiums are very slow-growing, producing but one leaf per year. Nevertheless, there are two approaches that permit the genetic and environmental components of variation to be distinguished in *Botrychium*. These are the Genus Communities Method and the Method of Mutual Associations (Wagner & Wagner, 1983a).

The Genus Communities Method is based on the tendency of congeneric species to grow together in the same habitats. These genus communities provide a natural common garden experiment: if problematic taxa maintain their differences consistently and persistently when growing together, it is evidence that their morphological differences are genetically fixed. The method was used successfully to differentiate *Botrychium hesperium* and *B. echo* (Wagner &

Wagner, 1983a, 1983b). Both species grow, often together, in the southern Rockies at elevations between 2,500 and 3,500 m, in rocky soil on grassy slopes, along roadsides, and at lake edges. The taxa are so similar that, although the original collectors noticed their differences, they did not initially recognize them as species. However, extensive fieldwork with large population samples demonstrated consistent differences in a number of gross- and micro-morphological characters and in phenology (Wagner & Wagner, 1983b). These differences were maintained where the species grew together, confirming that their distinctive characteristics are heritable. Further evidence for the reproductive isolation of *B. hesperium* and *B. echo* is provided by the sterility of the rare interspecific hybrids that are found at sites where the two species co-occur.

The basis of reproductive isolation between *Botrychium* species has only recently been elucidated. Studies of genetic variation in *Botrychium virginianum* (Soltis & Soltis, 1986), *B. dissectum* (McCauley et al., 1985), and *B. simplex* (W. Hauk, pers. comm.), which together represent all three North American subgenera, suggest that these species are selfers (intragametophytic self-fertilization, sensu Klekowski, 1969). Given that all *Botrychium* species have bisexual subterranean gametophytes, perhaps selfing is common in the genus, and represents the major isolating mechanism between homoploid species in genus communities.

The Method of Mutual Associations, closely related to the Genus Communities Method, is useful if the two taxa of interest do not grow together in the same place. In such cases, a third taxon is brought into the picture and is used as an assay for variation in the other two. The method is based on the principle that if taxon A grows together with taxon C in one place, and if taxon B grows with C in another place, and C is morphologically uniform from habitat to habitat, then the differences between A and B are probably genetically fixed (Wagner & Wagner, 1983a). The Method of Mutual Associations was used effectively to differentiate *B. mormo*, the little goblin fern, from the dwarf form of *B. simplex*, which it closely resembles. Because *B. mormo* and *B. simplex* do not occur together, *B. minganense*, which occurs with each, was used as the assay species and permitted the recognition of a number of genetically based differences between the two (Wagner & Wagner, 1983a).

In *Botrychium* subg. *Botrychium*, unlike the other examples given so far, the identification of subtle morphological differences between similar species preceded the demonstration of their reproductive isolation. Nevertheless, these moonworts meet the three criteria of cryptic species and may usefully be numbered among them.

C. The *Pityrogramma triangularis* complex

Cryptic species within the *Pityrogramma triangularis* (goldback ferns) complex of western North America are especially problematic because so far they appear to be morphologically indistinguishable. Alt and Grant (1960) made the first attempt to resolve biosystematic relationships within the *Pityrogramma*

triangularis complex (1960). Within *P. triangularis* var. *triangularis*, they found two quite similar yet morphologically separable diploids, which they called type A and type B. A previously unknown tetraploid taxon discovered in their sample was interpreted, on the basis of leaf morphology, as the derivative of a hybrid between types A and B. Chromosome pairing behavior in the tetraploid and backcross triploids suggested an allopolyploid origin for the tetraploid and indicated that types A and B were reproductively isolated (Alt & Grant, 1960).

Subsequent chemosystematic studies of *P. triangularis* flavonoids (Star et al., 1975a, 1975b; Smith, 1980) have shown that the situation in *P. triangularis* var. *triangularis* is even more complex than Alt and Grant recognized. Var. *triangularis* comprises an array of six distinct flavonoid chemotypes, two among the diploids and four among the tetraploids:

- | | |
|----------------|---------------------------------------------------------------------------------------------------------|
| 2X chemotypes: | ceroptin
kaempferol 4'-methyl ether |
| 4X chemotypes: | ceroptin
kaempferol 4'-methyl ether and 7,4'-dimethyl ether
kaempferol
galangin 7-methyl ether |

The partial to full sterility of inter-chemotype hybrids found in the study provides evidence for at least partial genetic isolation of the chemotypes at each ploidy level (Smith, 1980).

The extent to which morphological and phytochemical characters are correlated in the *P. triangularis* complex is so far unknown, as is the relationship between types A and B of Alt and Grant and the chemotypes of Smith. In consequence, it is unclear whether there are good field characters by which reproductively isolated taxa within var. *triangularis* can be recognized. The challenge to taxonomists posed by the cryptic entities in *Pityrogramma triangularis* was recognized both by Alt & Grant (1960) and by Smith (1980). The latter summarized the situation in these words:

"One dilemma met here is a common tormentor of vascular plant systematists: namely, how can one rationally treat taxonomically those members of a group which, even though they are reproductively isolated, nevertheless are distinguishable only by complex methodology generally beyond the reach of most people interested in identification, while at the same time imperfectly isolated entities receive formal taxonomic status because they possess superficially distinguishing marks."

We consider Smith's dilemma in the last section of this paper.

ARE CRYPTIC SPECIES COMMON IN THE FERNS?

At present it is not clear whether cryptic species are more common among ferns than other groups of organisms. The results of recent studies suggest that they are: in addition to the examples discussed above, cryptic species have also

been detected in *Cystopteris* (Haufler, pers. comm.), *Gymnocarpium* (Pryer & Windham, 1988), *Polypodium* (Haufler & Windham, 1988), and *Woodsia* (Windham, 1987). This flush of recent discoveries presents the impression that cryptic species are especially prevalent in ferns, but that impression may be an artifact of the recent incorporation of biosystematic data into systematic studies in the ferns. According to Mayr (1970), cryptic species are probably common in many groups of organisms and will be detected with increasing frequency as sensitive methods such as isozyme electrophoresis are applied to systematic problems.

Perhaps, on the other hand, cryptic species do occur more commonly in the ferns than other kinds of organisms. This may be because in the ferns there is no selection for visual recognition cues during speciation. Many angiosperm species are pollinated by animals that rely on visual cues for the recognition of species. Speciation may involve a shift from one pollinator to another and concomitant evolution of a new set of visual cues. In the ferns, however, animals have no role in the movement of gametophytes. Selection is not therefore expected to elicit the evolution of novel recognition characters in new species. Mayr (1963) summarized the relationship between the prevalence of cryptic species and the means of mate recognition in a group:

"Sibling species are apparently particularly common in those kinds of species in which chemical senses (olfactory and so on) are more highly developed than the sense of vision. Although indistinguishable to the eye of man, these sibling species are evidently dissimilar to each other, as is shown by cross-mating experiments. Sibling species are apparently rarest in organisms such as birds that are most dependent on vision in the role of epigamic characters."

Before the advent of biosystematics in pteridology, cryptic species were especially difficult to detect in ferns because in many lineages, such as *Adiantum* and *Botrychium*, reticulate evolution and phenotypic plasticity have blurred species boundaries. Also, because ferns have a relatively simple plant body, few structural characters are available for taxonomic analysis (Haufler, 1985, 1987). Thus the pteridologist working with prepared specimens may have less power to resolve problematic complexes than do specialists in other groups such as angiosperms.

CRYPTIC SPECIES AND TAXONOMIC PRACTICE IN THE FERNS

The examples above demonstrate that cryptic species are good biological and evolutionary species. They are not very satisfactory morphological species, however, because they are virtually indistinguishable using structural characters. How one treats cryptic species taxonomically, then, depends on one's species concept.

Whereas most investigators agree that a system of classification should reflect as nearly as possible the phylogenetic relationships of the taxa being classified, there has always been argument about the extent to which this objective is

possible, let alone practical. Although we recognize that they will not be applicable to every situation in the ferns, we present the following guidelines for the taxonomic treatment of cryptic species. We suggest that cryptic species represent independent evolutionary lineages and so deserve species names. At the same time, we acknowledge the need for a multipurpose classification, one useful for the herbarium curator, the conservation biologist, and the park naturalist, as well as the specialist. We recommend that reproductively isolated taxa be given species names if morphological characters have been found to differentiate them, even if the character differences are very subtle. Those investigators who have a specific interest in the group and can differentiate the cryptic entities will thereby have the means to communicate about them. For purposes of routine identification, however, specimens can be keyed out to species group, with an indication that two or more cryptic species may be represented in the sample (Ross, 1974; Grant, 1981; Wiley, 1981). In the case of species that are so far indistinguishable without recourse to special methodology, as in the chemotypes of *Pityrogramma triangularis*, species epithets may be superfluous. Although these taxa are evolutionary species, the practical problems of identifying specimens in such cases makes the names useless to all but the chemosystematist. Even though the taxa are unnamed, manuals and floras should note that biochemically differentiated species exist within the complex. It is probable that with continued study, characters will be found to separate the cryptic entities; we recommend that the species then be named.

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